Fruit quality assessment of watermelons grafted onto citron melon rootstock

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BACKGROUND

The grafting of watermelons (Citrullus lanatus) is a common technique that increases yields under stressful soil conditions. The most common rootstocks for watermelons are Cucurbita hybrids. However, they often have a negative impact on fruit quality. Exploiting novel Citrullus germplasm, such as citron melon (Citrullus lanatus var. citroides), is an alternative to avoid these quality problems.

RESULTS

Citron melon has been validated as watermelon rootstock, comparing its effects on watermelon quality to those of Cucurbita hybrids. Larger fruits with thicker rinds were observed in fruits from plants grafted onto both citron and Cucurbita rootstocks. The citron melon had no significant effect on flesh sugars or acid profiles compared to non-grafted watermelons, except for an increase in glucose and malic acid content, which also occurred in the Cucurbita rootstocks. The aroma profile of fruits produced onto citron melon was similar to that of the non-grafted and self-grafted controls. The citron rootstock didn't display the increased levels of (Z)-6-nonen-1-ol (a compound associated with pumpkin-like odors) found in fruits produced with Cucurbita hybrids.

CONCLUSION

The low impact of citron melon rootstock on fruit quality along with the enhanced resistance against nematodes, make the citron a promising alternative to Cucurbita rootstocks.

Keywords

Citrullus lanatus; fruit quality; grafting; aroma profile

INTRODUCTION

Grafting in watermelon (Citrullus lanatus (Thunb.) Matsum. & Nakai) production is a common technique that increases yields under stressful conditions.[1] It is mainly used to control Fusarium wilt, caused by Fusarium oxysporum Schlechtend Fr. F. sp. niveum (E.F. Sm.; W.C. Snyder and H.N. Hans), as well as other damaging soilborne diseases, like Verticillium wilt (Verticillium dahliae Kleb).[2] It is also an important management strategy that permits a faster response to variable scenarios of abiotic stress than conventional breeding. This technology was first used during the late 1920s in Japan and Korea, and was introduced to different European countries in the late 1980s and later to the USA.[3]

The most common commercial rootstocks for watermelons are Cucurbita interspecific hybrids (C. moschata Duchesne x C. maxima Duchesne) and bottle gourd accessions (Lagenaria siceraria Standl). These rootstocks confer resistance to most of the soilborne fungi affecting watermelon. However, they are susceptible to root-knot nematodes (RKN, Meloidogyne spp.).[4] These pathogens cause extensive damage to watermelon roots and increase the severity of Fusarium wilt in watermelon fields. RKNs used to be controlled in watermelon by fumigation with methyl bromide. However, the removal of methyl bromide from the markets has resulted in an increase of the impact of RKNs on watermelon and other cucurbit crops, as the alternative treatments are less effective than this fumigant.[5]

This situation has caused a spike in the search for resistances in other Cucurbitaceae genera that could lead to the development of alternative rootstock suitable for managing root-knot nematodes in watermelon crops. Some species belonging to the Cucumis genus (C. metuliferus E. Mey. ex Naud. and C. pustulatus Naudin ex Hook. f.) have been reported as being resistant to RKNs.[6] These rootstocks can be used for

watermelons, but are more suitable as rootstocks for other crops belonging to the same genus, such as melon and cucumber (C. melo L. and C. sativus L.)[7]. Other more promising materials for grafting watermelons are those belonging to Citrullus lanatus var. citroides (L.H. Bailey) Mansf ex Greb, also called citron melon. The citroides group is a group of ancient cultigens from Southern Africa that today can be found worldwide, and which is often considered to be an ancestor of cultivated watermelons.[8] Citron melons are cultivated around the world mainly for fodder and for the production of fruit preserves. Resistance to nematodes, expressed as less galling than that displayed by Cucurbita hybrids and bottle gourd rootstocks, has been reported in some citron melon accessions[5], suggesting the usefulness of this group as rootstock for managing RKNs in watermelon.[9] Some resistant accessions have already been validated in specific conditions,[10] giving higher yields in comparison with C. maxima x C. moschata commercial rootstocks. Whereas citron melons display lower levels of resistance to Fusarium than hybrid squash and bottle gourd rootstocks, watermelon plants grafted onto citron rootstocks showed enhanced tolerance to Fusarium wilt.[11] Rotation of citron melon with Cucurbita and Lagenaria rootstocks could be an effective practice in the combined management of nematodes and Fusarium.[9, 10]

Different studies have proven that rootstocks may influence, positively or negatively, scion fruit quality in many vegetables.[12,13, 14] The most obvious reason for a negative impact is rootstock/scion incompatibility. However, even in compatible combinations different effects are commonly observed. These effects are dependent on the rootstock-scion interaction, which can influence nutrient and water uptake, hormone synthesis, photosynthesis and other metabolic processes.[15] It has recently been described that rootstocks induce a differential gene expression in the scion, suggesting that this

mechanism may play an important role in mediating the physiological processes of grafted plants.[16]

Changes in fruit quality as a consequence of grafting can affect the external appearance and/or flesh characteristics, including the chemical composition and the organoleptic properties of the fruit. The most common effects on the external appearance of watermelons grafted onto Cucurbita hybrids or L. siceraria rootstocks are the increase in fruit weight, rind thickening and increased flesh firmness.[1, 17, 18, 19] Other clearly negative effects have been reported, such as more fibrous flesh,[20] poor color and taste, increased number of yellowish bands and the occurrence of hollow heart.[21] Changes in mineral composition, sugars, ascorbic acid, citrulline or lycopene contents also occur.[17, 18, 22, 23] The impact of grafting on fruit aroma has been studied to a lesser degree, although recent reports show an increase in the concentrations of certain aldehyde volatile compounds in watermelon fruits from plants grafted onto interspecific Cucurbita hybrids and Lagenaria rootstocks.[24] Most of these reported effects depend on the scion-rootstock combination. It is therefore imperative that we evaluate the impact of new putative rootstocks on scion fruit quality.

The aim of this study was to validate the use of a selected citron melon accession collected in Spain as rootstock for watermelon. This accession had been reported previously as highly resistant to nematodes.[25] The effect of this citron melon accession on fruit quality was studied and compared with non-grafted plants, self-grafted plants and plants grafted onto two Cucurbita hybrid rootstocks. The effect of grafting on both external and internal quality, including a detailed analysis of sugars, organic acids and volatile profiles was assessed.

EXPERIMENTAL

Plant Material

The Spanish accession Citrullus lanatus var citroides BGV0005167 held at the COMAV´s Genebank was assayed as a new potential rootstock for watermelon. This accession was collected in Ademuz (Valencia, Eastern Spain). It was tested against nematodes in natural conditions, and responded as highly resistant, with a significantly lower number of eggs and nematodes on roots compared to the watermelon scions.[25]

Its behavior as watermelon rootstock (coded as GC) was compared with two C. maxima x C. moschata hybrids (a new experimental F1 hybrid, coded GMM1, and the commercial F1 Cobalt from Rijk Zwaan, coded GMM2). The experimental hybrid was obtained by crossing the C. *maxima* accession VAV 1860 (Large Warted Hubbard from Australia) x the C. moschata PI 550689 (Canada Crookneck squash from USA). This parental combination was selected due to the high compatibility of the cross as well as the early flowering of the resulting hybrid. Non-grafted watermelons, coded NG, and self-grafted watermelons, coded SG, were included as controls. The watermelon F1 Oneida (Rijk-Zwaan) was used as scion in all treatments.

Field assay

The approach grafting method was used to graft 24 plants of the watermelon F1 Oneida onto each of the four rootstocks (citron melon, F1 experimental hybrid, F1 Cobalt and F1 Oneida). Once the grafting was consolidated (after 30 days), the plants were transplanted to an experimental field (a field belonging to the company Rijk Zwaan, located in Picassent, Valencia, Eastern Spain), along with 24 non-grafted plants of the F1 Oneida, used as non-grafted control.

The experiment was carried out in a randomized complete block design with four replications of each rootstock-scion combination and each control (each replication having six plants). Between-row and within-row spacing were 2.0 m and 1 m,

respectively. Plants were furrow irrigated and fertilized according to standard cultural practices. The six plants of each replication were used for fruit sampling. Twenty-four fruits were harvested per combination (six per replication) and were morphologically characterized. Sixteen fruits per combination (four from each replication) were additionally sampled for sugars, acids and aroma analysis. The harvesting of fruits was performed in two days and all fruits were characterized over a 5-day period. Fruits were harvested at visual maturity based on external indicators, such as senescent tendrils, ground spot color and external fruit color.

Fruit characterization

Each fruit (twenty-four per treatment) was characterized for the following traits: weight (g), length and width (cm), fruit shape (length/width ratio), rind and flesh thickness (mm and cm), rind and flesh firmness (kg cm^{-2}) (measured with a digital Penetrometer (8mm) FHT-803®, Melrose, MA), total soluble solids (quantified using a digital refractometer, Atago®, Tokyo, Japan), pH (measured with pH-indicator paper pH1-14 Merck, Darmstadt, Germany) and flesh color (measured with a colorimeter, Minolta CR-400, New Jersey, USA using the color parameters Hunter L, a and b).

A 5-cm cross section was obtained from the equatorial plane of each of four fruits per replication (sixteen fruits per treatment). Pericarp and approximately 2 mm of flesh and seeds were discarded. The remaining flesh was homogenized (KRUPS KB720, Groupe Seb Iberica, Barcelona, Spain) and kept frozen at -80ºC until metabolite analysis.

Reagents for metabolite analysis

Organic acid standards were prepared from their sodium salts or free acids. The chemicals used were of analytical grade and were purchased from Sigma (St. Louis, MO, USA). Glucose, fructose, sucrose and 2,6-pyridine dicarboxylic acid (PDC), hexadimethrine bromide (HDM) and sodium dodecyl sulphate (SDS) were supplied by

Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade and were used as received. Individual stock solutions of the analytes (1000 µg mL^{-1}) were prepared and stored at 4ºC until their use. Working solutions were prepared daily by diluting the stock solutions with deionized water.

Reference standards of volatile compounds were supplied by Supelco (Sigma-Aldrich and Fluka; Barcelona, Spain) as pure compounds (90 - 99.5% purity). Individual standard stock solutions (500 mg L^{-1}) were prepared in acetone. Then, several mixed standard solutions were obtained by 10-fold volume dilution in acetone. Calibration solutions were prepared from working solutions by consecutive volume dilutions with n-hexane to different final concentrations, according to the detector response for each compound (final concentrations ranging from 10 ng mL^{-1} to 5000 ng mL^{-1}). All standard solutions were stored at –18°C in sealed glass vials (without leaving any headspace) to avoid analyte losses and to ensure reproducibility. Gas Cromatography-grade solvents were obtained from Scharlab (Barcelona, Spain). SupelcleanTM ENVI-CarbTM 120-400 mesh, 500 mg SPE Tubes 6 mL (Supelco, Barcelona, Spain) were used as traps.

Analysis of sugars and acids

Fruit samples stored at -80ºC were thawed in a refrigerator in complete darkness. They were then centrifuged at 510 g for 5 minutes. The upper phase was diluted (1:20) in deionized water. The solution was filtered using centrifuge tube filters with 0.22 µm membranes (Costar® Spin-X®, Corning®, Amsterdam) and were subsequently analyzed.

The sugars sucrose, fructose and glucose, as well as malic, citric and glutamic acids were quantified by capillary electrophoresis following the method described previously[26, 27] on an Agilent 7100 system (Agilent Technologies, Waldbronn, Germany) using fused silica capillaries (Polymicro Technologies, Phoenix, AZ) with a

50-µm internal diameter, 363-µm external diameter, 67-cm total length, and 60-cm effective length. Capillaries were previously conditioned with the following flushes at 95,000 Pa and 50 C: NaOH 1 N (5 min), NaOH 1 N (5 min), and deionized water, Elix 3, Millipore, Billerica, MA (10 min). At the beginning of each working session, the capillary was flushed at 20ºC with the running background electrolyte (BGE) for 30 min. The BGE consisted of 20 mM 2,6-piridin dicarboxylic acid at pH 12.1 and 0.1% w/v hexadimethrine bromide. Between runs, the capillary was flushed with 58 mM SDS (2 min) and the running BGE (5 min). Samples were injected hydrodynamically at 3400 Pa for 10 s. Separations were performed at -25 kV and 20ºC. Indirect detection was done at 214 nm. Results are expressed in milligrams per kilogram of fresh weight (FW). Sucrose equivalents were calculated by multiplying sucrose, glucose and fructose contents by 1, 0.74 and 1.73, respectively, and then adding them up.[24]

Analysis of volatiles

Volatiles were extracted and analyzed following the methodology described previously[28]. The extraction was performed in a dynamic headspace system (DHS) using a homemade purge & trap device[29] using commercial (500 mg) SPE cartridges as a trap. Before analysis, the trap cartridges were conditioned with 5 mL of diethyl ether (Et_2O) and then by 5 mL of n-hexane, and were finally vacuum dried for 10 minutes. A sample amount of 30 g of homogenized fruit was weighed in a 150-mL flask closed with a glass cap with two connection tubes; the inlet tube was connected to a dry nitrogen gas (N_2) source and the outlet tube to the trap. Extraction was performed for 49 minutes with a nitrogen flow rate of 1.6 L min^{-1}. Samples were stirred at 7 g using a magnetic stir bar and heated to 40ºC.

After extraction, each cartridge was eluted with 5 mL of diethyl ether/hexane (1:1) followed by 5 mL of diethyl ether directly into a graduated glass tube. The extract was

evaporated under a gentle nitrogen stream at a controlled temperature of 35°C to a final volume of 0.5 mL. The final extract was divided into two aliquots in vials with 200 µL inserts, sealed and stored in a freezer at -20°C until their analysis by GC-MS (ion trap).

A Varian CP-3800 gas chromatograph coupled to a mass spectrometry detector (Saturn 4000, Varian) was used for the analysis of the volatiles included in Table 1. A 30 m×0.25 mm Supelcowax 10 (0.25 µm film thickness) capillary column was used for the separation, using helium at a constant flow of 1 mL min^{-1} as carrier gas. The temperature program was: 40°C for 5 min, then increased to 160 °C at 4 °C min^{-1}, and finally increased to 250 °C at 30°C min^{-1}, with a final isothermal stage of 1 min (total chromatographic analysis time 39 min). Injection of 1 µL of sample in the splitless mode (injection port temperature 220°C) was performed using a Varian 8400 autosampler equipped with a 10-µL syringe. MS (ion trap) determinations were performed in full scan mode (m/z scan range of 50 – 200 Da) using electron impact ionization (70 eV) in positive mode and external ionization configuration. GC-MS interface, ion trap and manifold temperatures were set at 275°C, 190°C and 60°C, respectively. The retention index for all studied compounds was calculated using a standard containing n-alkanes (C7-C30) on Supelcowax 10 capillary column and following the formula given by Kovats.[30]

Quantitation was carried out by means of external standard calibration curves obtained by using peak areas from the corresponding extracted ion chromatograms for the selected quantitation ion (Q) for each compound (Table 1). Compounds with concentrations exceeding the linearity range were quantified by diluting extracts with n-hexane until reaching the proper concentration.

Statistical analysis

Changes in fruit characteristics and sugar and acid contents were evaluated using ANOVA and LSD multiple range tests. The changes in the volatile composition of different watermelon genotypes due to grafting were evaluated jointly using multivariate analysis MANOVA and MANOVA biplot. In the MANOVA biplot subspace, the similarity between grafting treatments can be measured as an inverse function of their distance on the graph. The angle between variables can be interpreted as an approximation of their correlation. The inner product of a group marker with a variable marker approximates the mean of the kth group on the jth variable allowing for the characterization of the differences between groups. Univariate Bonferroni confidence circles are added to the group markers in such a way that the projections of the circles onto the direction representing a given variable represent an approximate confidence interval. The significance of the difference between groups with regards to a particular variable can be established by checking the overlap of their projections. The procedure is conservative in the sense that if no overlap is found it can be concluded that there is a significant difference, but if there is an overlap, a significant difference may be found along another direction in the multidimensional space. All MANOVA biplot calculations and graphs were made with MultBiplot, free-licensed software.[31]

RESULTS

Effect of grafting on fruit morphology and flesh properties

The comparison of fruits from non-grafted and self-grafted watermelon plants indicates that the grafting process itself does not alter fruit size and shape or essential flesh properties, such as firmness, soluble solids content and pH (Table 2). The only parameter affected by self-grafting was flesh color, with fruits from grafted plants having values for a and b color parameters that were significantly lower than those of

non-grafted plants, which means less intensity of the red and yellow flesh color components.

Unlike self-grafting, grafting onto non-watermelon rootstocks altered fruit size (Table 2). In fact, both types of rootstocks, citron melon (GC) and the two F1 Cucurbita hybrids (GMM1 and GMM2), increased fruit weight significantly (in comparison both to self-grafted and to non-grafted watermelons). This increment was 24% on average, and was associated with the production of wider and longer fruits, without significant fruit shape alterations. In addition, both citron melon and Cucurbita rootstocks yielded fruits with thicker rinds than SG and NG, which was also accompanied by an increase in flesh thickness (ranging from 16.9 to 17.2 in grafted versus 16.0 cm in non-grafted plants). These rootstocks resulted in fruits with firmer flesh than those produced by self-grafted plants.

The citron melon was the only rootstock that significantly increased the total soluble solids content compared to SG and NG watermelons (10.2 versus 9.3 and 9.6 Brix degrees). Of all the fruits harvested from grafted plants, the fruits from GC plants had the most similar flesh color to the non-grafted watermelons. In fact, unlike the fruits from self-grafted plants and from plants grafted onto the Cucurbita hybrids, this rootstock did not cause a reduction in the values of the Hunter color parameters.

Effect of grafting on flesh sugar and acid content

As occurred with fruit morphology and flesh properties, we did not find significant differences in acid contents between fruits of non-grafted and self-grafted plants (Table 3). However, some significant changes in these metabolites were found in fruits from plants grafted onto non-watermelon rootstocks. Both citron melon and Cucurbita hybrids significantly increased the malic acid content compared with non-grafted plants (Table 3). Citron melon did not alter the citric and glutamic acid contents in comparison

to fruits from non-grafted and self-grafted plants, whereas fruits from plants grafted onto Cucurbita hybrids had lower contents of these acids than those of citron melon-grafted plants.

Fructose content was not affected by grafting (Table 2). However, the citron rootstock was the non-watermelon rootstock that produced fruits with the highest glucose amounts, significantly higher than fruits from NG plants, which is consistent with the high value of total soluble solids content that was observed in fruits from GC plants. Sucrose measurements were more variable. No significant differences were observed between non-grafted plants and those using either citron melon or the commercial Cucurbita rootstock. Only the experimental Cucurbita hybrid rootstock significantly reduced sucrose contents. Similar results were observed with sucrose equivalents.

Effect of grafting on flesh aroma profile

Of the 61 volatiles analyzed, 32 were found in quantifiable amounts (Table 4). The following twenty-nine compounds were analyzed, but their presence was not detected in the samples: (Z)-3-hexen-1-ol acetate, ethyl hexanoate, 1-decanol, 2-methyl propyl acetate, 2-hydroxy-benzaldehyde, amyl acetate, benzyl acetate, butyl acetate, butyl isobutyrate, diethyl carbonate, ethyl heptanoate, ethyl pentanoate, ethyl-(E)-2-butanoate, ethyl-3-(methylthio) propanoate, eucalyptol, eugenol, guaiacol, heptyl acetate, hexyl acetate, isoamyl butyrate, isobutyl butyrate, linalool, methyl butyrate, methyl hexanoate, methyl-2-methyl butyrate, phenol, butyl butyrate, phenylethyl acetate and propyl butyrate.

Considering the complexity of the analysis, a MANOVA biplot (Fig.1) was selected in order to elucidate the precise differences in the profile of aroma volatiles. The individual samples of each combination were highly clustered in the biplot and separated from those of the other combinations. The MANOVA confirmed the

significance of the effect that the rootstock had on the aroma volatile profile of watermelon (Roy's greatest root test, $P<10^{-3}$). The first axis explained 43% of the variance and the second 31%. The Bonferroni circles whose projections on the vector of a certain compound do not overlap represent statistically significant differences.

Few differences in the volatile profile were found between the non-grafted and the self-grafted controls, which was consistent with the limited effect of self-grafting as described earlier (Fig. 1). Fruits from NG and SG plants stand out for their elevated accumulation of several compounds, including those deriving from the carotenoid degradation pathway, such as geranylacetone, 6-methyl-5-hepten-2-one, beta-ionone and beta-cyclocitral (Table 4).

Significant effects on flesh volatile profiles were observed in fruits from plants grafted onto the Cucurbita hybrids (GMM1 and GMM2) and GC (Fig. 1) compared to the controls. These grafting combinations showed circles that do not overlap among themselves nor with NG or SG, indicating different aroma profiles depending on the rootstock. The greatest differences to NG and SG were found in fruits from plants grafted onto the Cucurbita hybrids. In general, low accumulation of volatile compounds, with high amounts of (Z)-6-nonenal, (E-Z)-2-6-nonadien-1-ol and (E-Z)-2-6-nonadienal, were observed in fruits from GMM1-grafted plants (Fig 1, Table 4), whereas the GMM2 rootstock resulted in higher accumulation of several alcohols, including 1-nonanol, 1-octanol, 1-hexanol and (Z)-3-nonen-1-ol. Both Cucurbita hybrids stood out for their accumulation of (Z)-6-nonen-1-ol. In fact, the vector for this compound represented the most important differences from the controls.

Watermelon fruits from GC plants showed a more similar volatile profile to the SG and NG controls, with an intermediate position in the MANOVA biplot between the controls and GMM1 (Fig. 1). The differences between the controls and the CG

rootstock were related to the higher accumulation of (Z)-6-nonenal, (E)-2-nonenal, (Z)-6-nonen-1-ol, (E-Z)-2-6-nonadienal and (E,E)-2-4-heptadienal as well as the lower accumulation of carotenoid-derived volatiles in fruits from plants grafted onto the citron melon rootstock (Fig 1, Table 4).

DISCUSSION

Fruit quality is a complex trait that involves external and internal parameters that are determined to satisfy the consumers' preferences. In watermelon, the main quality-related properties are fruit morphology and flesh texture, color, flavor and aroma. Since fruit quality can be negatively modified by grafting,[12] it is important to evaluate for these traits each new rootstock-scion combination, such as that of citron melon-watermelon. The use of citron as rootstock is very promising due to its high level of resistance to nematodes.[9,10]

In this paper, we have shown that citron melons cause a range of effects in watermelon fruits that are also observed in plants grafted onto Cucurbita hybrids, but have less impact than these commonly used rootstocks on certain quality traits.

The common effects of citron and Cucurbita rootstocks are mainly related to the production of bigger watermelon fruits. Better yields associated with both larger fruits as well as the increase of fruit number per plant have often been reported for watermelons grafted onto Cucurbita hybrids, in both Fusarium-infested and non-infested soils.[24] Only a few reports have studied these effects in watermelon plants grafted onto citron rootstocks, and have indicated an increase in yield in citron melon-grafted watermelons cultivated in soils infested with nematodes, but they do not clearly report an increase in fruit size.[9,10]

In agreement with what is commonly found in watermelon grafting reports,[12,19,32] the changes that we observed in fruit size did not cause important fruit shape alterations,

although they were associated with the thickening of the watermelon rind.[18,19] In our assay, the nearly 2-mm increase in rind thickness was associated with a higher flesh content (about 1 cm wider flesh), which minimized the negative impact of this alteration.

Another change often reported as a consequence of grafting watermelons onto different common Cucurbita interspecific hybrids is the increase in flesh firmness.[1,17] This is an important trait as it is related to fruit quality and might also influence postharvest behavior. Flesh firmness is an indicator of ripeness in watermelon. In the current study, all experimental plots were harvested in two days, when the fruits of all combinations showed external indicators of maturity.[33] The fact that we did not find significant differences in fruit firmness between either the Cucurbita or the citron-grafted watermelons and the fruits from non-grafted plants may suggest that we harvested fruits at a similar maturity state, which is essential in order to assess and compare the quality of the fruits from the different treatments.

The development of flesh color might also be associated with the ripening stage. Variable effects of rootstocks on watermelon flesh color have been reported.[22,34,35] Our results showed a certain reduction in the redness and yellowness of flesh color, which affected both the self-grafted and the Cucurbita-grafted plants, whereas the citron melon rootstocks resulted in a more similar color profile to that of the non-grafted control.

Citron melons seem to affect fruit acidity and sweetness less negatively than Cucurbita hybrids. The balance between flesh sweetness and acidity represents a central parameter in determining fruit flavor. Even though all non-watermelon rootstocks increased malic acid concentrations, the citric and glutamic acid contents of the fruits harvested in citron melon-grafted plants were similar to those of the fruits from non-grafted plants, whereas Cucurbita hybrids reduced the content of the two acids. There are limited studies on the

modification of watermelons' acidic profile as a consequence of grafting, and most indicate higher titratable acidity in fruits from grafted plants.[35, 36]

Regarding the effect on sugar content, our results are in agreement with those found by Colla et al. [32] and Soteriou et al. [35] who found similar sugar compositions in fruits from grafted and non-grafted plants. The relative sweetness of fructose is greater than that of sucrose,[27] which makes the fact that grafting had no effect on fructose content quite favorable, as there were higher concentrations of fructose than glucose and sucrose in all combinations. However, we found a non-desirable effect of grafting, as it reduced the sucrose content. This effect was variable in the Cucurbita hybrids, as it only affected the fruits collected from plants with the experimental Cucurbita rootstock, whereas the commercial Cobalt did not decrease sucrose levels compared to non-grafted plants. Despite this sucrose reduction, none of these Cucurbita rootstocks differed from non-grafted plants in sucrose equivalents. Sucrose equivalents are calculated in order to correct the concentration of each sugar with its sweetening power. This variable has been found, in other crops,[27] to be more correlated with sweetness perception than sugar concentration. Therefore, and as was previously stated for other watermelon-Cucurbita hybrid combinations, it remains unclear if reductions in specific sugars will really be perceived by consumers if no differences are detected in sucrose equivalents.[36] Previous studies also show variable results, reporting both reductions and no effects in sugar content as a consequence of grafting in Cucurbita hybrids and Lagenaria rootstocks, depending on the environment, ploidy level of the scion and stage of fruit development at harvesting.[1,12,18] Our study shows that the fruits harvested from plants grafted onto citron melon rootstocks retain the highest levels of all sugars, in addition to the highest level of sucrose equivalents, similar to those found in the non-grafted plants.

Even though grafting in watermelon has been practiced since 1920, there is almost no information available on the effect of rootstock on the aroma profile. Recently, Petropoulos et al.[24] reported the effect of grafting onto TZ 148 (C. moschata x C. maxima hybrid) and Dias F1 (Lagenaria hybrid) on the aroma profile of two varieties of watermelon used as scion (Obla F1, seeded watermelon, and Vanessa F1: unseeded mini watermelon). The use of the Cucurbita rootstock increased the levels of the seven volatiles they quantified in the seeded watermelon, including (Z)-6-nonenal, nonanal, (E,Z)-2,6-nonadienal, (Z,Z)-3,6-nonadien-1-ol and (E)-2-nonenal.

In our work, a much higher number of volatiles were quantified, including four of those studied by Petropoulos et al.[24] ((Z)-6-nonenal, (E)-2-nonenal, nonanal, (E,Z)-2,6-nonadienal). Two of these compounds ((Z)-6-nonenal and (E,Z)-2,6-nonadienal) have been found to be among the most abundant volatiles in seedless watermelons, and have been associated with melon-like and cucumber-like aromas, respectively.[37] The higher values of these compounds found in fruits from plants grafted onto Cucurbita hybrids were interpreted by Petropoulos et al.[24] as a negative effect of these rootstocks on the volatile profile of watermelons.

Our results also clearly suggest that the rootstock used has an important effect on the volatile profile of the watermelons produced. We also found increased amounts of the two compounds that confer melon- and cucumber-like aromas in the experimental Cucurbita hybrid, but not in the commercial rootstock, which had similar values to that of self- and non-grafted plants.

The volatile profiles of fruits harvested from plants grafted onto Cucurbita hybrids most notably show the highest levels of (Z)-6-nonen-1-ol. This compound confers pumpkin-like odors,[38] which is detrimental to fruit quality. In fact, in sliced watermelon, the concentration of (Z)-6-nonen-1-ol increases, and it has been suggested that it may be

partially related to the pumpkin-like off-odors and squash-like off flavors often attributed to overripe whole watermelon.[39] This effect could be a consequence of a higher accumulation of these compounds, which are specific to the Cucurbita rootstocks, or possibly to a faster ripening process in these grafted plants. Considering that these fruits showed similar values of several ripening-related parameters (including rind and flesh firmness, pH and sugars content) to the control fruits, the first explanation may be the most probable. Also, as King et al.[2] suggested, the "squash" flavor may arise when the fruits are harvested too soon, but in our case, the occurrence of external signals of fruit ripening and pH and sugar values do not support this explanation.

Fruits harvested from plants grafted onto citron melons also have high amounts of the volatile compounds associated with melon- and cucumber-like aromas ((Z)-6-nonenal and (E,Z)-2,6-nonadienal). However, they have much lower amounts of the squash-like aroma-associated compound (Z)-6-nonen-1-ol than fruits from Cucurbita hybrids.

One of the main differential characteristics of the aromas of non-grafted and self-grafted watermelons is that they have high levels of geranyl-acetone, 6-methy-5-hepten-2-one and beta-cyclocitral in comparison with the other rootstocks. These compounds originate from the degradation of carotenoids in tomato and watermelon.[40,41] Specifically, 6-methy-5-heten-2-one and geranylacetone seemingly derive from lycopene and other noncyclic tetraterpenoids while beta-ionone and beta cyclocitral derive from beta-carotene.[42]

This result correlated with the observed differences in fruit color, as the presence of these volatiles has been related to total carotenoid and lycopene contents in watermelon.[43] In fact, the experimental Cucurbita hybrid with the strongest impact on flesh yellowness (in b parameter) had lower levels of these carotenoid-derived volatiles. However, this does not explain the differences observed in flesh redness (a parameter)

between fruits from non-grafted and self-grafted plants. Fruits from GC plants have only slightly lower amounts of these compounds than non-grafted and self-grafted watermelons, and are similar to those of the plants grafted onto the commercial Cucurbita hybrid.

Overall, grafting watermelon cultivars onto resistant rootstocks is a highly recommendable practice as it enhances the field performance of the scions, but the negative impact on fruit quality can prevent the use of some rootstock-scion combinations. Our results confirm the occurrence of various effects on fruit quality, including a relevant impact on aroma profile, which is dependent on the rootstocks, thereby confirming the existence of variation within the Cucurbita hybrids and the differential effect of citron melons. The use of the citron melon rootstock from the same Citrullus genus produces a more similar volatile profile to that of non-grafted plants. To our knowledge, this paper is the first to describe the effect of citron melon rootstock on watermelon quality properties such as acid, sugar and aroma profiles. The similar volatile profile produced as compared to the controls seems to indicate that citron melon would be a good alternative to the classic Cucurbita hybrids

CONCLUSIONS

The use of citron melon rootstock may represent an interesting alternative to the classic Curcurbita hybrids. The use of this rootstock compared to non-grafted watermelon has a limited effect on the sugar and acid profiles of watermelons or may even improve them slightly. On the other hand, the aroma volatile profile, when using citron melon rootstock, is more similar to the non-grafted control than the commonly used Cucurbita hybrids. Additionally, the use of Cucurbita hybrids increases (Z)-6-nonen-1-ol, which may be detrimental to watermelon flavor, an effect not observed in citron melon. The variability detected in the aroma profiles of different Cucurbita hybrids also suggests

that the effect of rootstock on the aroma profile should be considered in rootstock breeding programs.

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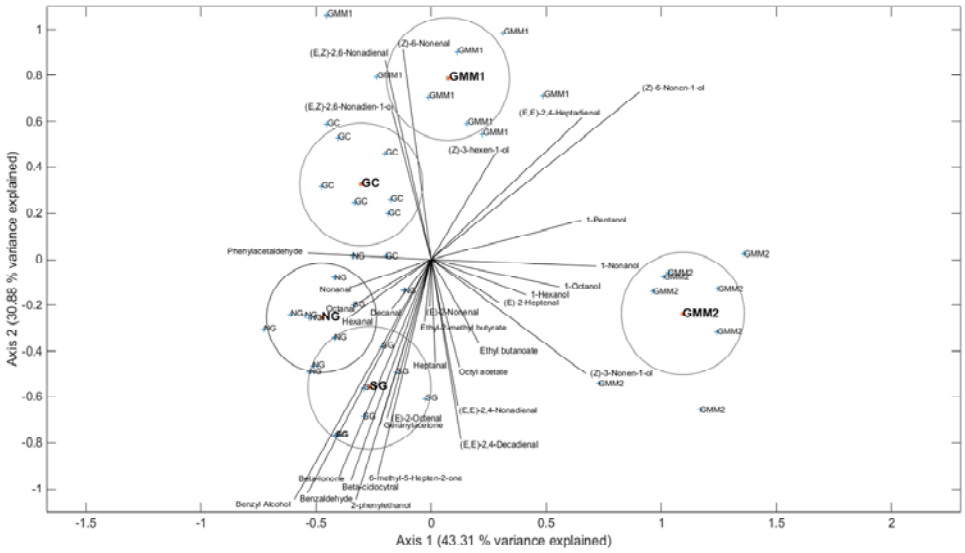

Fig. 1. MANOVA biplot of fruit volatiles. Circles represent Bonferroni confidence intervals for the mean value of each material. In all the cases the scion corresponds to cv. 'Oneida RZ'. NG: 'Oneida RZ' non-grafted; SG: Self-grafted; GC: grafted on citron melon; GMM1: grafted on experimental Cucurbita F1 hybrid; GMM2: grafted on 'Cobalt RZ' a commercial Cucurbita F1 hybrid.

Table 1. Volatiles analyzed in the watermelon samples.

* CAS = Chemical abstract service

**Chromatographic parameters (retention time (Rt), retention index (RI) and quantitation ion (Quan Ion)) obtained from GC-MS chromatograms. P&T extraction using EnviCarb 500 mg cartridges, 30 g of watermelon sample. Retention index calculated with n-alkanes on Supelcowax 10 (bonded polyethylene glycol) capillary column.

***Linearity range corresponding to the real concentration of standards used for calibration.

Table 2. Characteristics of watermelon fruits (Citrullus lanatus F1 Oneida) harvested from non-grafted plants (NG), self-grafted plants (SG) and plants grafted onto the experimental *Cucurbita* (C. maxima *x* C. moschata) hybrid F1 (GMM1), the commercial *Cucurbita* hybrid Cobalt (GMM2) and one new experimental citron melon (Citrullus lanatus var citroides) (GC).

*Different letters in the same row indicate significant differences (LSD, P=0.05)

Table 3. Sugar and acid concentrations of watermelon fruits (Citrullus lanatus cv F1 Oneida) harvested from non-grafted plants (NG), self-grafted plants (SG) and plants grafted onto the experimental Cucurbita (C. maxima *x* C. moschata) hybrid F1 (GMM1), the commercial *Cucurbita* hybrid Cobalt (GMM2) and one new experimental citron melon (Citrullus lanatus var citroides) (GC).

*Different letters in the same row indicate significant differences (LSD, P=0.05)

Table 4. Mean contents of the volatiles quantified in watermelon fruits (Citrullus lanatus cv F1 Oneida) harvested from non-grafted plants (NG), self-grafted plants (SG) and plants grafted onto the experimental *Cucurbita* (C. maxima *x* C. moschata) hybrid F1 (GMM1), the commercial *Cucurbita* hybrid Cobalt (GMM2) and one new experimental citron melon (Citrullus lanatus var citroides) (GC).

*Values expressed in ng g^{-1} fresh weight.

Table 1

Volatiles analysed in the watermelon samples.

#	Compound name	CAS° nº.	MW	Rt (min)**	RI (SupelcoWax 10)**	Quan Ion (m/z)**	Linearity range (ng mL^{-1})***	r^2
1	Methyl butyrate	623-42-7	102	4,04	900	43	454 - 2268	0,988
2	Methyl-2-methylbutyrate	868-57-5	116	4,55	921	88	23 – 2270	1,000
3	2-methyl propyl acetate	110-19-0	116	4,67	926	43	111 - 2765	1,000
4	Ethyl butyrate	105-54-4	116	5,34	947	71	265 – 2648	0,997
5	Ethyl-2-methyl butyrate	7452-79-1	130	5,80	963	57	44 – 2173	0,995
6	Butyl acetate	123-86-4	116	6,51	985	43	105 - 2616	0,999
7	Hexanal	66-25-1	100	7,20	993	56	743 – 7425	0,995
8	Propyl butyrate	105-66-8	130	8,24	1034	43	55 - 2745	1,000
9	Ethyl pentanoate	539-82-2	130	8,70	1047	88	25 - 2508	0,999
10	Butyl isobutyrate	97-87-0	144	9,13	1059	89	22 - 2155	0,999
11	Isobutyl butyrate	539-90-2	144	9,58	1071	71	30 - 2950	1,000
12	Ethyl-(E)-2-butanoate	623-70-1	114	9,69	1074	69	26 – 2596	0,999
13	Amyl acetate	123-86-4	116	10,11	1086	43	116 - 2900	1,000
14	Heptanal	111-71-7	114	10,49	1096	70	55 - 2720	1,000
15	Methyl hexanoate	106-70-7	130	10,58	1099	74	26 - 2643	1,000
16	Eucalyptol	470-82-6	154	10,99	1111	93	21- 2083	1,000
17	Butyl butyrate	109-21-7	144	11,74	1131	71	60 - 2965	0,988
18	Ethyl hexanoate	123-66-0	144	12,32	1147	88	87 - 2183	1,000
19	1-pentanol	71-41-0	88	12,91	1162	55	128 - 3195	1,000
20	Isoamyl butyrate	106-27-4	158	13,46	1178	70	26 - 2640	1,000

21	Hexyl acetate	142-92-7	144	13,73	1188	43	30 - 3038	1,000
22	Octanal	124-13-0	128	14,27	1200	69	50 – 2479	0,998
23	(Z)-3-hexen-1-ol, acetate	3681-71-8	142	15,30	1231	67	27 – 2660	1,000
24	(E)-2-heptenal	18829-55-5	112	15,48	1235	83	85 - 2118	1,000
25	Ethyl heptanoate	106-30-9	158	15,88	1247	88	30 - 2980	1,000
26	6-methyl-5-Hepten-2-one	110-93-0	126	15,99	1251	109	21 – 4260	0,999
27	1-hexanol	111-27-3	102	16,54	1267	56	205 – 4100	1,000
28	Heptyl acetate	112-06-1	158	17,27	1288	43	30 – 2975	1,000
29	(Z)-3-hexen-1-ol	928-96-1	100	17,57	1297	67	51 – 5050	0,992
30	Nonanal	124-19-6	142	17,90	1307	57	108 – 5420	0,999
31	(E)-2-octenal	2548-87-0	126	19,05	1343	55	33 – 833	0,999
32	(Z)-6-nonenal	2277-19-2	140	19,75	1366	81	61 - 12260	0,988
33	Octyl acetate	112-14-1	172	20,60	1391	43	58 - 2913	1,000
34	(E,E)-2,4-heptadienal	881395	110	21,17	1408	81	22 - 2203	1,000
35	Decanal	112-31-2	156	21,31	1413	67	25 – 2469	0,999
36	(E,E)-2,4-hexadienoic acid, ethyl ester	2396-84-1	140	21,58	1423	67	24 – 2370	0,999
37	Benzaldehyde	100-52-7	106	21,97	1436	105	26 – 2613	1,000
38	(E)-2-nonenal	18829-56-6	140	22,43	1450	81	49 – 9780	0,982
39	Linalool	78-40-6	154	22,89	1465	93	23 - 2345	0,998
40	1-octanol	111-87-5	130	23,14	1474	55	41 - 2063	0,999
41	Ethyl-3-(methylthio)propanoate	13327-56-5	148	23,37	1483	74	32 - 3185	1,000
42	(E,Z)-2,6-nonadienal	557-48-2	138	23,95	1501	70	95 - 9460	0,996
43	β-ciclocytral	5392-40-5	152	24,82	1533	110	24 - 2358	0,996
44	Phenylacetaldehyde	122-78-1	120	25,59	1559	91	23 - 2260	0,941
45	1-nonanol	143-08-8	144	26,15	1585	55	41- 4140	0,999
46	2-hydroxybenzaldehyde	90-02-8	122	26,66	1594	122	29 - 2915	0,997
47	(Z)-3-nonen-1-ol	10340-23-5	142	26,80	1600	67	24 – 4750	1,000
48	(E,E)-2,4-nonadienal	5910-87-2	138	27,32	1618	81	22 - 2155	0,997
49	(Z)-6-nonen-1-ol	35854-86-5	142	27,75	1634	67	37 – 7455	0,999
50	Benzyl acetate	140-11-4	150	28,04	1646	108	13 - 1250	1,000
51	1-decanol	112-30-1	158	29,01	1680	55	83 - 2075	0,996
52	(E,Z)-2,6-nonadien-1-ol	28069-72-9	140	29,15	1685	67	22 - 2183	0,999
53	(E,E)-2,4-decadienal	25152-84-5	152	30,30	1727	81	22 – 2180	0,996
54	Phenethyl acetate	103-45-7	164	30,38	1734	104	25 – 2452	1,000

55	Geranylacetone	689-67-8	194	31,44	1773	43	44 - 4345	0,997
56	Guaiacol	90-05-1	124	31,59	1779	109	26 – 2638	1,000
57	Benzyl Alcohol	100-51-6	108	31,99	1795	79	13 – 2500	0,999
58	2-phenylethanol	60-12-8	122	32,85	1830	91	13 - 1250	0,999
59	β-ionone	14901-07-6	192	33,49	1856	177	24 - 2363	0,999
60	Phenol	108-95-2	94	35,24	1937	94	54 – 2173	0,988
61	Eugenol	97-53-0	164	36,99	2098	164	27 - 2650	0,997

* CAS = Chemical abstract service

**Chromatographic parameters (retention time (Rt), retention index (RI) and quantitation ion (Quan Ion)) obtained from GC-MS chromatograms. P&T extraction using EnviCarb 500 mg cartridges, 30 g of watermelon sample. Retention index calculated with n-alkanes on Supelcowax 10 (bonded polyethylene glycol) capillary column.

***Linearity range corresponding to the real concentration of standards used for calibration.

Table 2

Characteristics of watermelon fruits (Citrullus lanatus F1 Oneida) harvested from non-grafted plants (NG), self-grafted plants (SG) and plants grafted the experimental Cucurbita (C. maxima *x* C. moschata) hybrid F1 (GMM1), the commercial Cucurbita hybrid Cobalt (GMM2) and one new experimental citron melon (Citrullus lanatus var citroides) (GC).

	Non-grafted NG	Self-grafted SG	Cucurbita F1 experimental GMM1	Cucurbita F1 Cobalt GMM2	Citron melon GC
Fruit weight (g)	3157.8a*	3321.7a	3958.7b	4160.2b	3970.9b
Fruit length FL (cm)	20.5a	21.1a	21.2ab	22.5b	22.4b
Fruit width FW (cm)	17.6a	17.9a	18.8b	19.2b	18.8b
Fruit shape (FL/FW)	1.16ab	1.17b	1.12a	1.17b	1.19b
Rind Thickness (mm)	7.8a	7.9a	9.8b	10.1b	9.3b
Flesh Thickness (cm)	16.0a	16.3ab	16.9bc	17.2c	17.0bc
Rind Firmness (kg cm^{-2})	12.37a	12.89a	12.98a	12.65a	12.92a
Flesh Firmness (kg cm^{-2})	1.21ab	1.04a	1.38b	1.27b	1.38b
Total Soluble Solids TSS (ºBrix)	9.6ab	9.3a	9.6ab	10.1bc	10.2c
pH	5.2ab	5.3b	5.1ab	5.2ab	5.1a
Hunter L	30.6c	29.1abc	28.1ab	27.8a	30.1bc
Hunter a	21.7b	19.6a	20.8ab	20.9ab	22.4 b
Hunter b	10.5c	9.5ab	9.1a	9.5ab	10.1bc

*Different letters in the same row indicate significant differences (LSD, P=0.05)

Table 3

Sugars and acids concentrations of watermelon fruits (Citrullus lanatus cv F1 Oneida) harvested from non-grafted plants (NG), self-grafted plants (SG) and plants grafted the experimental Cucurbita (C. maxima x C. moschata) hybrid F1 (GMM1), the commercial Cucurbita hybrid Cobalt (GMM2) and one new experimental citron melon (Citrullus lanatus var citroides) (GC).

	Non-grafted (NG)	Self-grafted (SG)	Cucurbita F1 experimental (GMM1)	Cucurbita F1 Cobalt (GMM2)	Citron melon (GC)
Malic acid (mg kg-1)	163.0a*	195.1ab	219.9b	220.1b	230.5b
Citric acid (mg kg-1)	41.9bc	52.7bc	22.6a	37.1ab	53.3c
Glutamic acid (mg kg-1)	2.2abc	3.6bc	0.5a	0.9ab	4.1c
Fructose (mg kg-1)	2411.6a	2696.6a	2305.6a	2558.0a	2814.8a
Glucose (mg kg-1)	1630.9a	1919.9ab	1773.8ab	1894.6ab	2083.4b
Sucrose (mg kg-1)	1185.4b	1651.3c	688.3a	1118.1ab	1155.5b
Sucrose equivalents (mg kg-1)	6564.3ab	7737.2b	5989.7a	6945.4ab	7566.8b

*Different letters in the same row indicate significant differences (LSD, P=0.05)

Table 4

Mean contents of the volatiles quantified in watermelon fruits (Citrullus lanatus cv F1 Oneida) harvested from non-grafted plants (NG). self-grafted plants (SG) and plants grafted the experimental Cucurbita (C. maxima x C. moschata) hybrid F1 (GMM1), the commercial Cucurbita hybrid Cobalt (GMM2) and one new experimental citron melon (Citrullus lanatus var citroides) (GC).

#	Compound name	Non-grafted (NG)	Self-grafted (SG)	Cucurbita F1 experimental (GMM1)	Cucurbita F1 Cobalt (GMM2)	Citron melon (GC)
1	(E)-2-Heptenal	4.7*	4.4	3.7	6.0	5.8
2	(E.Z)-2.6-Nonadienal	284.2	226.5	382.6	243.2	367.2
3	(Z)-3-hexen-1-ol	23.5	29.2	46.6	32.9	22.8
5	(Z)-6-Nonenal	94.6	89.1	160.1	97.0	173.2
7	(E)-2-Nonenal	501.6	413.4	347.0	504.2	559.1
8	(E)-2-Octenal	7.2	5.8	3.5	6.1	6.4
9	(E.E)-2.4-Decadienal	4.1	3.1	1.4	4.3	3.6
10	(E.E)-2.4-Heptadienal	2.5	2.3	3.2	3.7	3.6
11	(E.E)-2.4-Nonadienal	2.6	2.6	1.3	3.0	2.8
12	(E.Z)-2.6-Nonadien-1-ol	24.4	16.2	37.0	14.9	18.4
13	(Z)-3-Nonen-1-ol	1251.9	1366.7	1096.4	1928.0	1122.7
14	(Z)-6-Nonen-1-ol	53.1	49.5	130.9	131.9	70.4
16	1-Hexanol	60.0	76.7	74.3	88.7	48.9
17	1-Nonanol	170.3	175.2	212.0	291.7	154.0
18	1-Octanol	14.3	15.9	16.0	19.4	14.1
19	1-Pentanol	25.0	24.1	27.8	31.6	25.8
22	2-phenylethanol	1.1	1.4	0.4	0.9	1.0
23	6-methyl-5-Hepten-2-one	53.1	50.8	29.8	45.3	42.6

25	Benzaldehyde	3.4	3.3	1.0	2.1	2.7
26	Benzyl Alcohol	16.6	15.8	8.8	11.3	12.1
28	β-Ionone	4.0	3.6	1.8	2.8	2.8
29	β-cyclocitral	1.2	1.1	0.5	0.9	0.8
32	Decanal	5.2	4.5	4.1	4.3	4.3
34	Ethyl butanoate	50.3	42.3	36.1	54.3	34.3
38	Ethyl-2-methyl butyrate	7.1	7.6	6.8	6.8	5.8
42	Geranylacetone	134.9	143.2	85.0	120.4	125.7
44	Heptanal	3.4	3.3	2.6	3.5	3.5
46	Hexanal	104.1	109.8	82.4	90.4	120.5
54	Nonanal	207.8	268.0	194.1	189.6	284.0
55	Octanal	7.2	9.1	6.2	6.3	9.3
56	Octyl acetate	0.9	0.5	0.2	0.8	0.2
58	Phenylacetaldehyde	0.7	0.6	0.5	0.1	0.6

*Values expressed in ng g^{-1} fresh weight.